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The Synthesis of Photoswitchable Triptan Derivatives

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THE SYNTHESIS OF PHOTOSWITCHABLE TRIPTAN DERIVATIVES

by

Chelsea Sainsbury

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Chemistry)

The Honors College

University of Maine

May 2021

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ABSTRACT

Serotonin has various functions throughout the body and directly effects many neurological diseases/disorders, like depression, that are linked to the dysregulation of serotonin. Triptans are indole containing drugs that bind to a subset of serotonin receptors (5-HT_{1B} and 5-HT_{1D}) and are used to treat migraines. In this project, the synthesis of an indole intermediate is attempted. Ideally, an azobenzene would have been added to the 5th position (replacing the primary amine). Azobenzenes are compounds composed of 2 benzene rings connected by a nitrogen-nitrogen double bond that can switch between cis and trans conformations by absorbing different wavelengths of light. The transformation of the indole intermediate into a photoswitch is believed to be possible based on the structures of triptans. The first two steps of the synthesis of the indole intermediate were completed and product was produced with 72.9% yield. The column conditions that provided the best separation was determined to be 5% methanol in DCM with triethylamine. The reaction stopped working due to age and instability of the reagents.

ACKNOWLEDGEMENTS

I would like to thank my family, for without them I would not have made it this far. I would also like to thank my friends for always having my back and making sure I didn't overwork myself. I would like to thank Dr. Kienzler for allowing me to take on this project and for showing support over these past four years. I also would like to thank the Kienzler research group for creating a nice lab atmosphere and helping me with any questions I had. I need to thank Dr. Gramlich for allowing me to finish my research in his lab and for answering my numerous questions. Lastly, I would like to thank the Gramlich research group for creating an exciting lab environment and for welcoming me into the lab for the short time I was there.

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CHAPTER I

INTRODUCTION

Serotonin

Serotonin, or 5-hydroxytryptamine (5-HT) (Figure 1), is a neurotransmitter that has a variety of functions throughout the body. It is present in the heart, the respiratory system, the intestines, the central nervous system, and in the reproductive organs¹. Some of its functions are regulating heart rate, platelet aggregation, mood, sleep, etc. The dysregulation of serotonin in the body has been linked to diseases such as irritable bowel syndrome (IBS), nausea and emesisⁱ, pulmonary arterial hypertensionⁱⁱ (PAH), sudden infant death syndrome (SIDS), mood disorders, pain disorders, and preeclampsia. Since the same serotonin receptors are in different parts of the body and regulate different biological processes, there has been a longstanding need to design drugs to target the specific receptor in the correct tissue that is responsible for the diseases. To address this challenge, we must talk about the structures of the serotonin receptors.

ⁱ Vomiting.

ⁱⁱ High blood pressure that affects the arteries and heart.

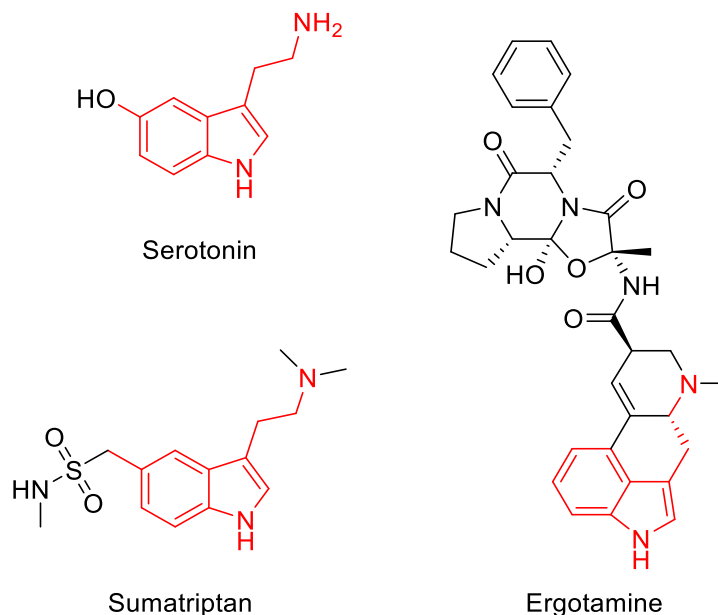


Figure 1. Structures of 5-hydroxytryptamine (serotonin), sumatriptan, and ergotamine. Sumatriptan is an example of a triptan, drugs that are used to treat migraines and ergotamine is a natural product that binds to the 5HTRs. Triptans bind to the 5HT_{1B} and 5HT_{1D} receptors. The structure of serotonin (red) is seen in both sumatriptan and ergotamine.

In humans, there are 7 serotonin receptor families composed of 15 serotonin receptor (5-HTR) subtypes¹. The families are the 5-HT₁ receptors to the 5-HT₇ receptors. All of the receptors are G protein-coupled receptors (receptors that interact with G proteins², which transmit signals from stimuli outside the cell to inside) except 5-HT₃, which is a ligand-gated ion channel (membrane proteins that allow certain ions to cross the plasma membrane³). The 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} receptors are located in the CNS and the blood vessels. Whereas the 5-HT_{1F} receptor is located only in the CNS⁴. The 5-HT₁ and 5-HT₅ receptors both negatively couple with adenylyl cyclase and when activated, they lower the level of cyclic adenosine monophosphate (cAMP)⁵. Through the Gq signaling pathway, the 5-HT₂ receptors increase the amount of inositol triphosphate and diacylglycerol, causing Ca²⁺ to be released⁵. The 5-HT₄ and 5-HT₇ receptors increase cyclic

AMP activity⁵. The 5-HT₃ receptor is a sodium/potassium selective cation channel and causes depolarization of the plasma membrane⁵.

The serotonin receptors have similar binding pockets because serotonin must be able to bind to them. These similarities can cause problems, like unforeseen side effects of a drug due to binding to a different receptor than the one it's supposed to target. A well-known example of this is Fen-Phen, fenfluramine-phentermine (Figure 2). Fen-Phen was designed to be an anti-obesity drug combination, that was supposed to cause appetite suppression from phentermine releasing norepinephrine into the body⁸. However, it was pulled from the market due to norfenfluramine (the metabolite of fenfluramine) activating the 5-HT₂ receptors⁹, causing heart valve disease.

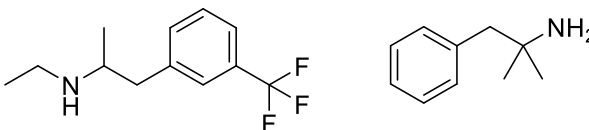


Figure 2. Structures of fenfluramine (left) and phentermine (right), the two constituents of Fen-Phen, the anti-obesity drug combination.

The serotonin receptors are also selective as to what binds to them. When looking at the crystal structures of the 5-HT_{1B} and 5-HT_{2B} receptors (Figure 3), it can be seen that they are comprised of seven helices^{10,11}. These helices interact with compounds that bind to the receptor. However, these two receptors have different active site conformations¹⁰. Which means that although they both have the same natural ligand, serotonin, another agonist for the 5-HT_{1B} receptor might not be the best agonist for the 5-HT_{2B} receptor. Where an agonist is a drug that binds and activates a receptor and an antagonist is a drug that binds to a receptor to block it, not activate it. Medicinal chemists are currently trying

to synthesize drugs that will target individual receptors. Doing so will decrease the risk of side effects from the drugs binding to other receptors (like with Fen-Phen).

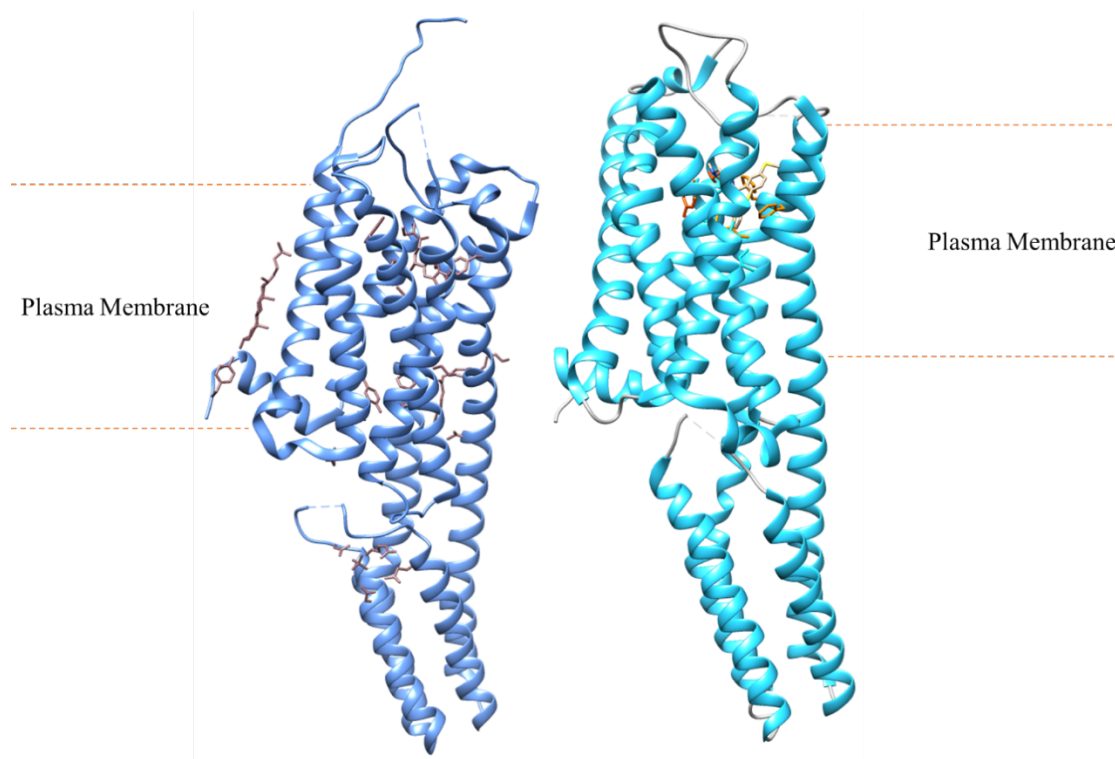


Figure 3. The 3-D structures of 5-HT_{2B} receptor (5TVN⁶) (left) and the 5-HT_{1B} receptor (5V54⁷) (right) as reported by the Protein Data Bank (PDB)

Triptans

Triptans are drugs that bind to a subset of serotonin receptors (5HT_{1B} and 5HT_{1D}) and are used to treat migraines¹². It is believed that when triptans bind to the 5HT_{1B} receptors, they cause vasoconstriction of the enflamed cranial arteries that occur during migraines and when they bind to the 5HT_{1D} receptors they reduce pain¹³. Throughout the years, the synthesis of triptans has been optimized to increase the yield, the bioavailability (the amount of a drug that causes an effect once brought into the body), and better the pharmacokinetics (the movement of and any changes to a drug while in the body) (see Table 1).

<i>Triptan</i>	Oral bioavailability (%)	t_{1/2} (h)	Year Released
<i>Sumatriptan</i>	15	2	1995
<i>Zolmitriptan</i>	40-48	2.5-3	1997
<i>Naratriptan</i>	63-74	5-6.3	1998
<i>Rizatriptan</i>	45	2-3	1998
<i>Almotriptan</i>	80	3.2-3.7	2001
<i>Frovatriptan</i>	24-30	25	2001
<i>Eletriptan</i>	50	3.6-5.5	2002

Table 1. The Pharmacokinetics of seven triptans along with the year they were released¹²

The structure of triptans, as seen in Figure 4, all have the same tryptamine center, but they differ at the 3rd and 5th positions. These differences in structure are why each triptan interacts differently with the 5HTRs and has different pharmacokinetic properties (i.e. bioavailability). This is seen when comparing sumatriptan and almotriptan, they have the same 3rd position, but different 5th positions. Then when comparing their pharmacokinetic properties, sumatriptan is more bioavailable, has a shorter half-life, etc. than almotriptan. The same trend in bioavailability, half-life, etc. is seen with the other triptans. When the 5th or 3rd position varies, so do the pharmacokinetic properties.

A future goal of this project is to attach an azobenzene to the 5th position on the indole intermediate. The hypothesis is that product produced from this will still retain its agonistic characteristics based on the structures of triptans. This is due to triptans retaining agonistic activity while containing various groups attached to the 5th position of the indole (i.e. donitriptan). Docking studies looked into this 5th position and the binding of triptans to the 5-HT_{1B} and 5-HT_{2B} receptors. The study found that triptans readily bound to the 5-HT_{1B} receptor but had to conform to unfavorable positions to bind to the 5-HT_{2B} receptor¹¹. This is due to the narrower V-shaped binding pocket in the 5-HT_{2B} receptor¹¹. The study's results indicate that the wider V-shape of the 5-HT_{1B} receptor is key to incorporate the various groups at the 5th position in triptans¹¹.

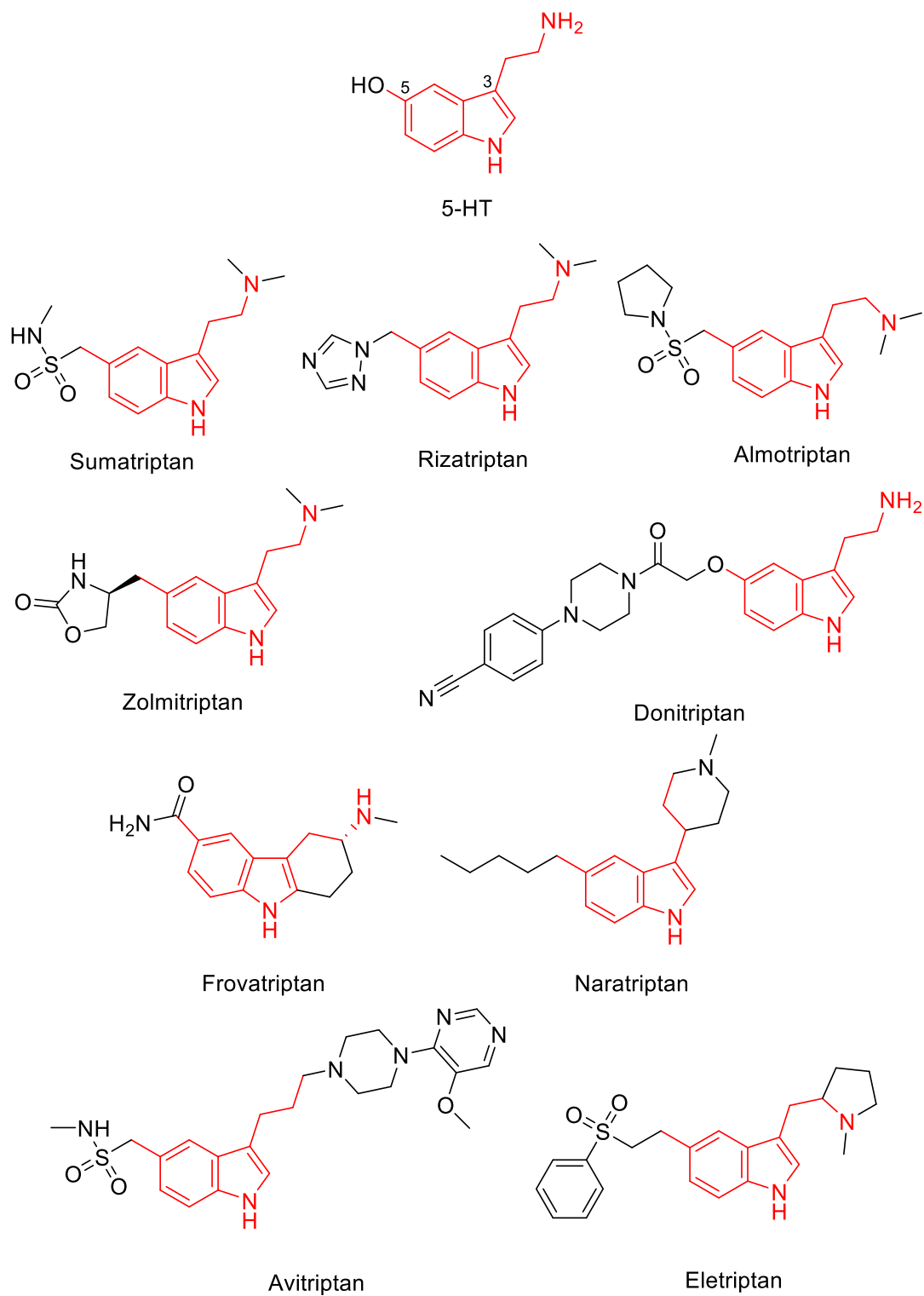


Figure 4. The various different structures of triptans as compared to 5-HT (shown in red).

Photopharmacology

Photopharmacology is a subset of pharmacology that uses light to control biological functions¹⁵. In this process, light is used to activate or deactivate a drug using moieties known as photoswitches. A photoswitch is a compound whose structure changes (photoisomerizes) with the application of light. There are a variety of photoswitches (Figure 5) that can be used for controlling biological functions, such as azobenzenes, diarylethenes, stilbenes, spiropyrans, thiophenefulgides, hemithioindigos, etc.

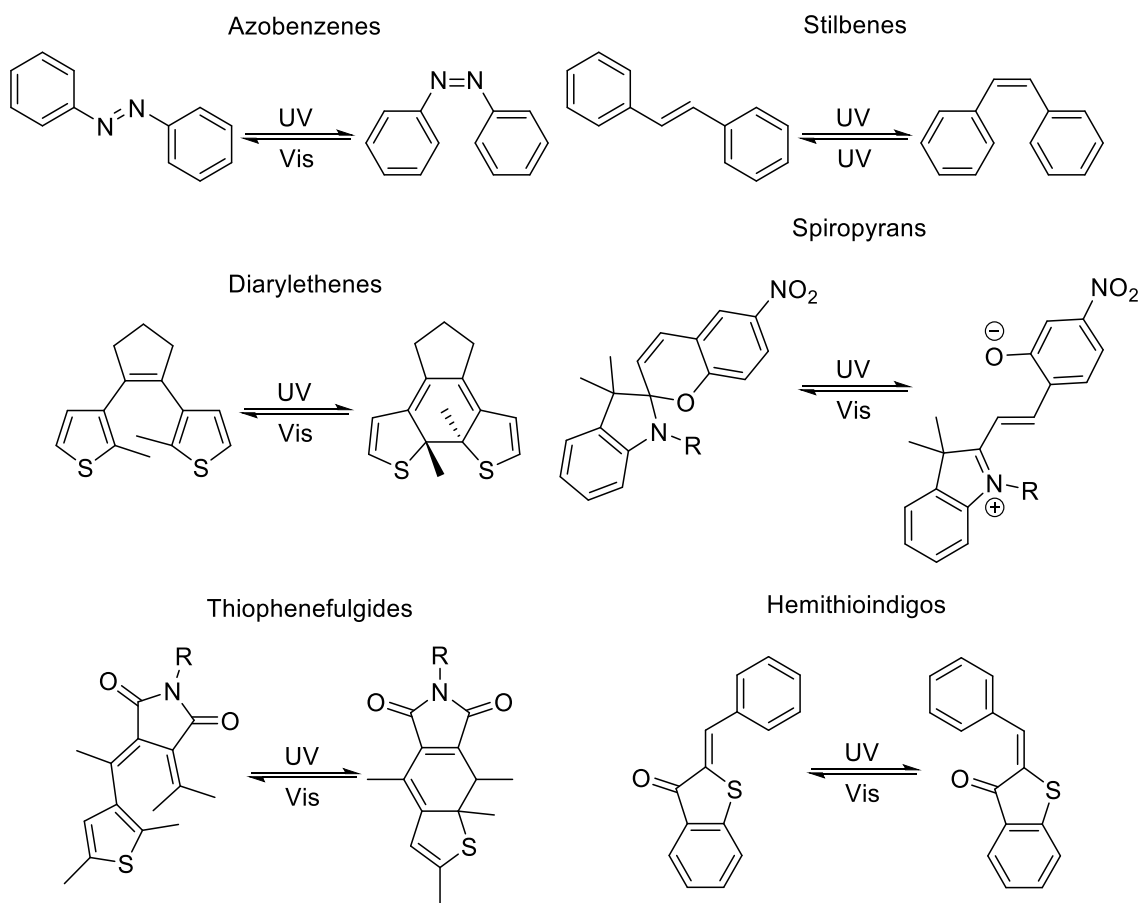
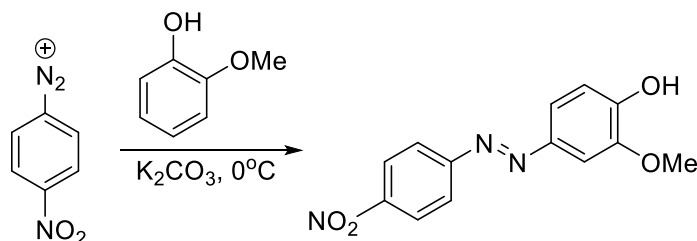


Figure 5. Structures of some of the common photoswitches

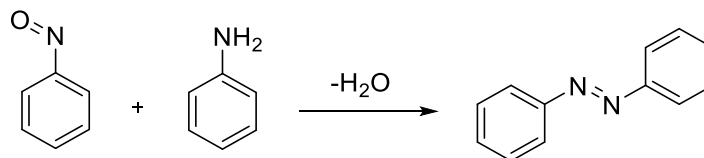
Azobenzenes undergo reversible cis-trans photoisomerization using UV-visible light or heat¹⁶. Stilbenes also undergo photoisomerization, but of the E-Z conformations

and it is not always reversible¹⁶. Spiropyrans undergo the cleavage of a C-O bond with irradiation using UV light to produce the merocyanine form¹⁶. This process is also reversible using light and heat. Diarylethenes also undergo reversible photoisomerization, however it is caused by light induced cyclization due to the presence of a hexatriene group¹⁶. These photoswitches are not thermally reversible. Thiophenefulgides are similar to diarylethenes in how they undergo reversible light induced cyclization of the hexatriene group forming a cyclohexadiene ring¹⁶. Hemithioindigos undergo photoisomerization from the Z-E conformations of the olefinic bond¹⁶. These photoswitches undergo a variety of light driven reactions, such as double bond isomerization, 6 pi electrocyclization, etc.

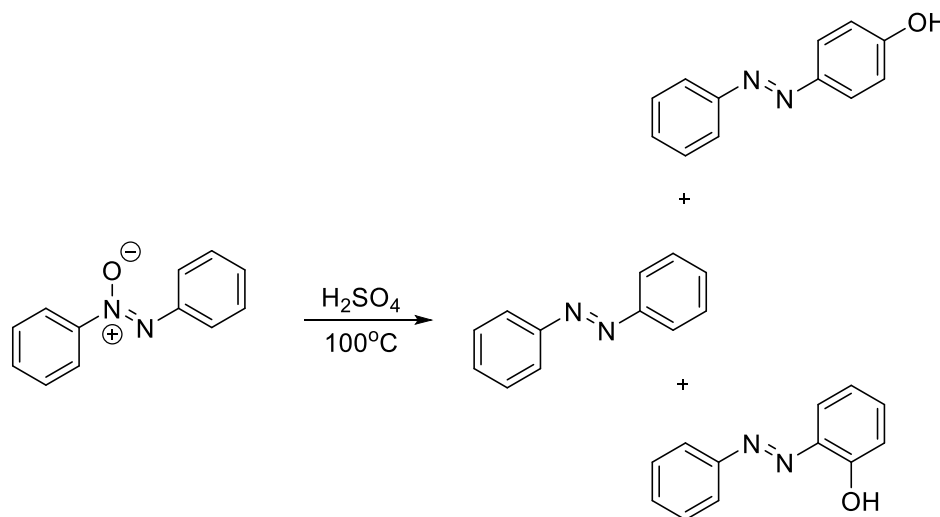
Azobenzenes are compounds composed of 2 benzene rings connected by a nitrogen-nitrogen double bond. They can be synthesized through azo coupling (Scheme 1), the Mills reaction (Scheme 2), the Wallach reaction (Scheme 3), etc¹⁷. In azo coupling, a primary amine is diazotized (becomes a diazonium salt) and undergoes an electrophilic substitution of an aromatic moiety¹⁷. In the Mills reaction an aniline attacks a nitroso-aromatic moiety under acidic conditions to produce an azobenzene¹⁷. In the Wallach reaction, an azoxybenzene is converted into 2- or 4-hydroxy substituted azobenzene moiety under acidic conditons¹⁷.



Scheme 1. The azocoupling reaction to produce an azobenzene



Equation 2. The Mills reaction of nitrosobenzene with an aniline to produce an azobenzene.



Scheme 3. The Wallach reaction to produce 3 azobenzenes.

These compounds are able to switch between cis and trans conformations using light (Figure 6). The formation of the cis isomer occurs with irradiation of approximately 320 nm of light and the formation of the trans isomer occurs through either thermal relaxation or irradiation with approximately 460 nm of light¹⁶. However, the substitution on the benzenes can greatly change the wavelengths. The trans conformation is more stable than the cis due to steric interactions present in the cis conformation. This ability is valuable because it can be used for drug delivery¹⁷, to alter the properties of materials¹⁸, and to be used in polymers to release materials from micelles¹⁹. We predict that the azologizationⁱⁱⁱ of the indole intermediate is possible based on the structures of triptans. Ideally, the triptan

ⁱⁱⁱ The transformation of substance into an azobenzene containing photoswitch.

derivative synthesized will bind to a serotonin receptor in either the trans- or cis- configuration, but not both (Figure 7), resulting in the photocontrol of the receptor.

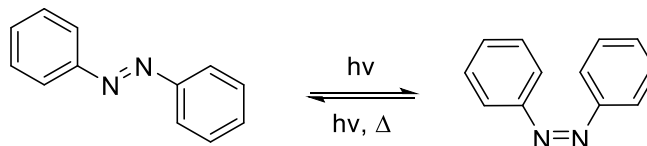


Figure 6. The structure of an azobenzene switching between the trans and cis conformations

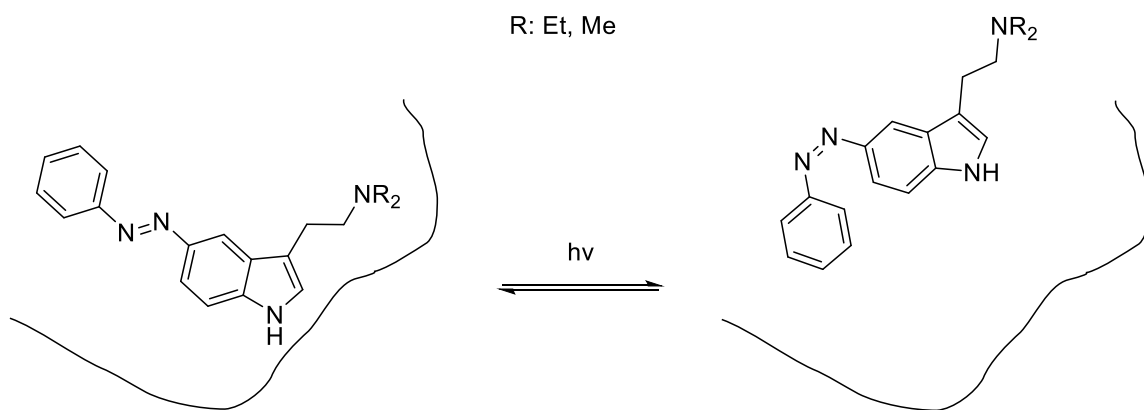
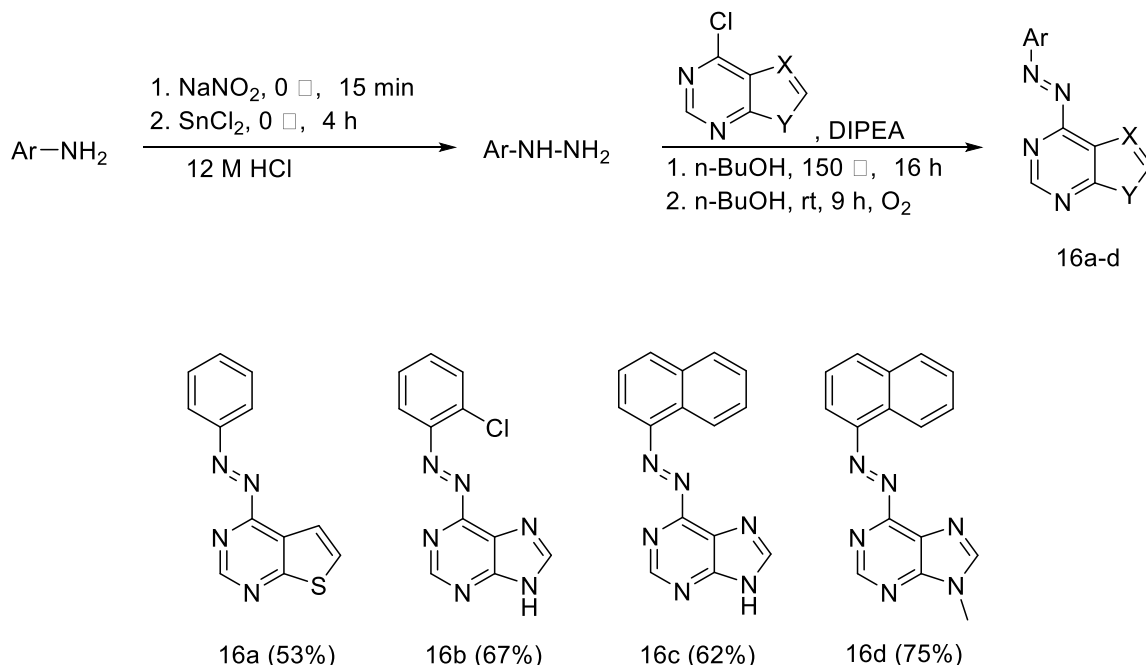


Figure 7. Schematic showing theoretical binding (left) and unbinding (right) of the photoswitch containing triptan derivative when it is exposed to UV light.

Currently there has been the photocontrol of the 5-HT_{2C}²⁰ receptor (2013) and the 5-HT₃²¹ receptor (2019). For the photocontrol of the 5-HT_{2C} receptor, an optogenetic solution was created as a possible treatment for anxiety²⁰. The optogenetic solution created was a melanopsin based probe to activate the Gq signals in the 5-HT_{2C} receptor domains²⁰. In the case of the 5-HT₃ receptor, nine antagonists were reviewed, however only one kept its antagonistic activity while being a photoswitch²¹. The antagonist synthesized that kept its antagonistic activity (16c) can be seen in Scheme 4. The photoswitch used for this antagonist was an azobenzene. Its ability to remain a photoswitch while retaining its

antagonistic is believed to be due to the potency of the starting structure for the synthesis of 16c.



Scheme 4. The scheme for the synthesis of four of the 5-HT antagonists synthesized by Rustler et. al.²¹ where 16c is the only photoswitch that retained antagonistic activity. Where DIPEA is diisopropylethylamine.

Motivation

This research is pertinent because more than 264 million people worldwide have depression²², 1 billion people suffer from migraines²³, about 10-15% of the population has IBS²⁴, about 15 people per million people are diagnosed with PAH²⁵, and as of 2017 there were 1,400 deaths in the US due to SIDS²⁶. These are all serotonin related diseases that people currently suffer from. Researching the function of the different serotonin receptors is necessary for medical applications to be developed. Thus, the photocontrol of serotonin receptors could clarify their role in many important biological processes.

The purpose of this project is to synthesize and photochemically characterize a series of light activated triptan derivatives (Figure 8) to control the 5-HT_{1B/D} receptors. This

will be done by synthesizing an indole compound with an aniline on the 5th position. Ideally this aniline would have to be replaced by an azobenzene and then exposed to UV light to test the optical and agonistic characteristics. However, due to complications during the first half of the synthesis and time constraints, this was unable to be completed. Despite this, the first two steps of the synthesis were completed, relatively pure product was collected (based on NMR), ideal column conditions were determined, and a plan for future work is proposed.

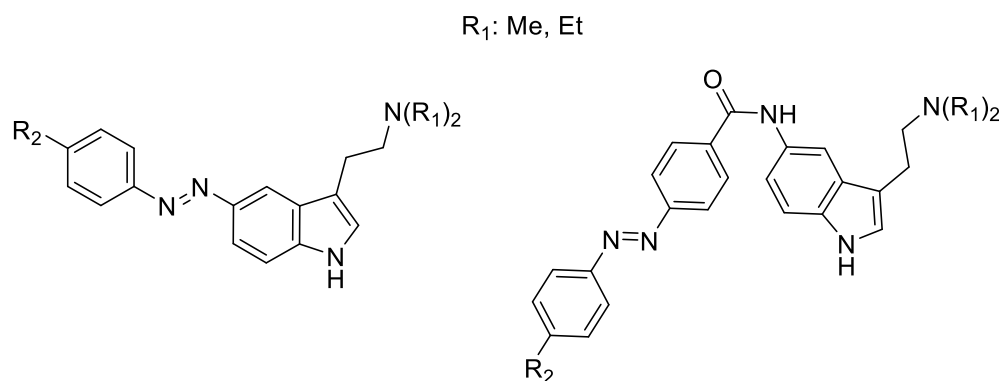


Figure 8. The two targeted chemical structures that would have been synthesized in this project. Substitution of the R_2 group can be varied to change the photochemical properties of the photoswitch.

CHAPTER 2

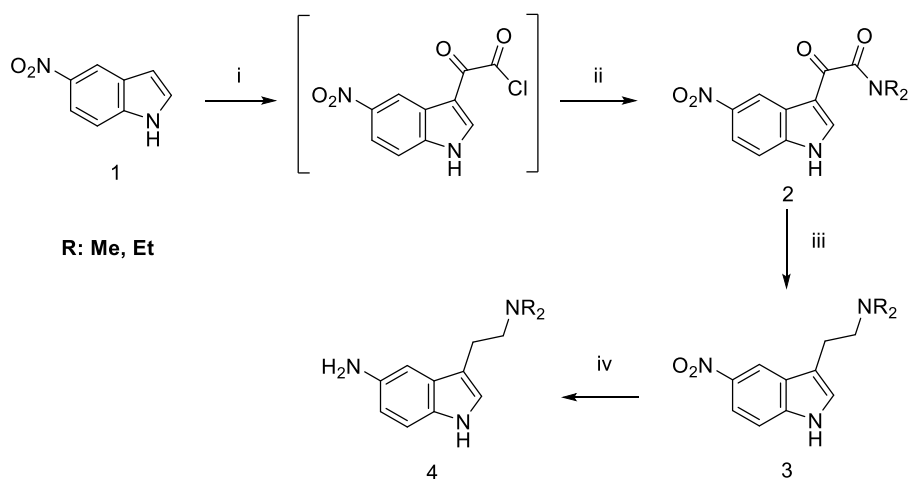
METHODS AND MATERIALS

Reagents and Equipment

The solvents and reagents used in the synthesis of the indole intermediate are 5-nitroindole, phthalimide, oxalyl chloride, diethylamine, dimethylamine, borane tetrahydrofuran, hydrogen, palladium on carbon, and magnesium sulfate (used for drying) purchased from Sigma Aldrich and Fischer Chemicals. The solvents used in the reactions, work ups, and purification include acetone, dichloromethane, diethyl ether, triethylamine, methanol, sodium bicarbonate, hydrochloric acid, hexanes, DI water (from local system), deuterated acetone, deuterated chloroform, and deuterated methanol purchased from Sigma Aldrich and Fischer Chemicals.

The equipment used for the synthesis of the indole intermediate include Heidolph Heizbad Hel-VAP rotary evaporator, a Yamato rotary evaporator, a Mettler Toledo balance, a Sartorius balance, a Chemglass Life Sciences AREX 6 Digital Pro hot and stir plate, a Fisher hot and stir plate, and a Bruker 500-megahertz (MHz) nuclear magnetic resonance (NMR) spectrometer.

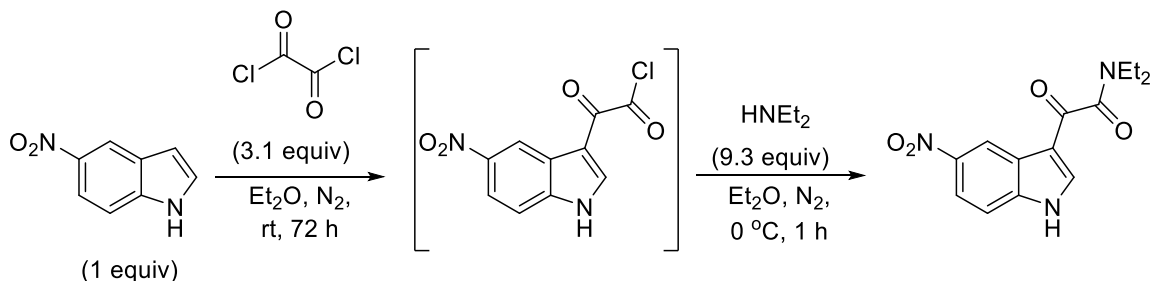
Synthetic Methods



Scheme 5. The reaction scheme for the synthesis of the indole intermediate, 4, based on a published route²⁷. Reagents: i. oxalyl chloride, diethyl ether, phthalimide, rt, 72 h. ii. diethylamine or dimethylamine, diethyl ether, 0 °C, 1 h. iii. BH₃-THF, rt, 16 h, CsF, Na₂CO₃, reflux, 16 h. iv. H₂, Pd/C, ethanol, rt, 6 h.

The reaction scheme for the synthesis of the indole intermediate can be seen in Scheme 5. The production of compound two was successfully completed for both the diethylamine and dimethylamine reactions. The reduction of the carbonyls on compound 2 to product compound 3 was attempted once. The specific reactions are listed in the following sections.

Synthesis of Indole Intermediate with diethylamine



Scheme 6. Diethylamine reaction to produce compound 2

5-nitroindole 30.9 mg (0.1906 mmol) was added to a 25 mL pear flask. Next, 2 mL of diethyl ether were added to the pear flask. The flask was then flushed with nitrogen and then 0.11 mL (1.28 mmol) of oxalyl chloride was added to the flask. The contents were stirred for 72 hours at room temperature. Reaction progress was monitored using TLC using 10% acetone in DCM, 5% methanol in DCM, and 5% methanol in DCM with triethylamine. After 72 hours, 1 mL of diethyl ether was added to the flask. The reaction was then placed in an ice bath and flushed with nitrogen while 0.18 mL (1.74 mmol) of diethylamine were added. The reaction was stirred at 0 °C overnight. The resulting yellow mixture was extracted using DCM and water three times. The reaction was then washed with ethyl acetate and water three times. The reaction underwent column chromatography in 5% methanol in DCM with triethylamine. The fractions were collected and NMR was run in deuterated methanol. The reaction was then washed three times with sodium bicarbonate and then washed with brine three times. The solution was concentrated in a rotary evaporator and high vac to produce a yellow/orange solid with 72.9% yield. A second NMR was taken in deuterated acetone.

In the following reactions, the sodium bicarbonate wash was done during the work up along with drying using magnesium sulfate. Also, residual amine was present in liquid form in collected product after purification using column chromatography. To remove this, the product was concentrated on the rotary evaporator for a longer period of time or the product was left on high vacuum. However, high vacuum caused product degradation, resulting in the discontinuation of that method. This was seen in the ^1H NMR taken before and after high vacuum of a sample. There were unknown aromatic impurities seen in the spectrum before high vacuum that had an integration of approximately 0.3 (Figure 9). After

high vacuum, the integrations of the impurities had increased to approximately 0.4 (Figure 10), indicating that the product had degraded.

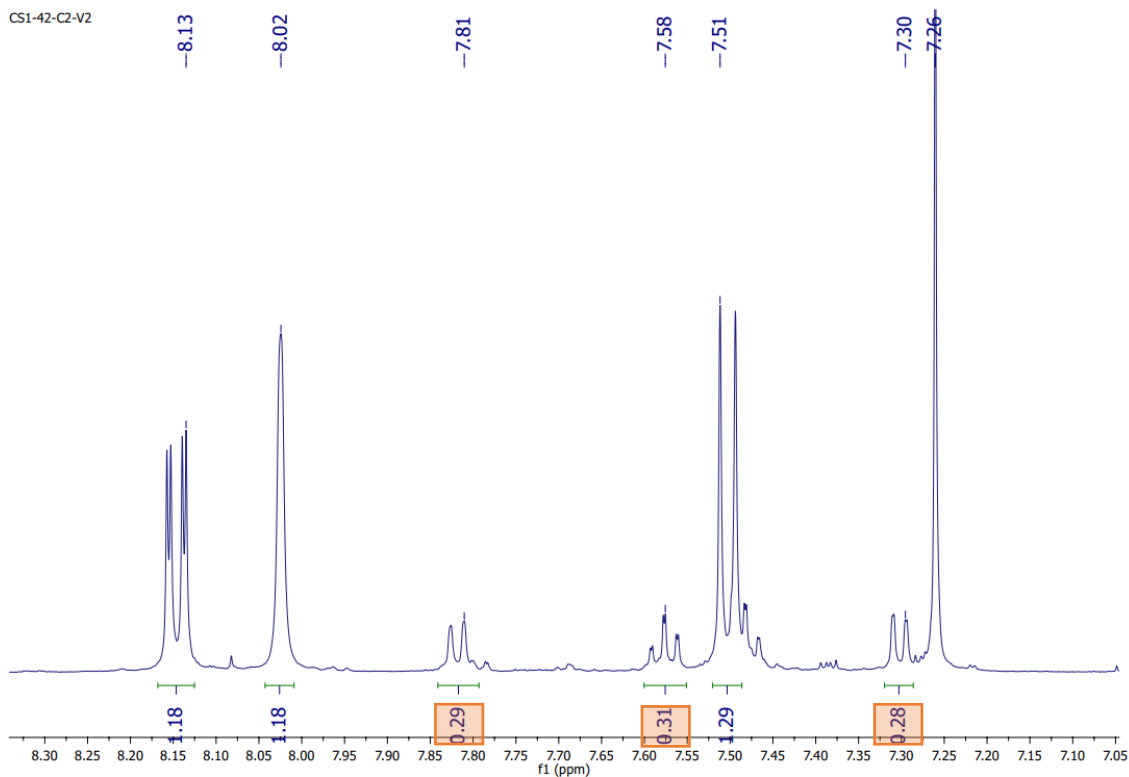


Figure 9. Zoomed in ^1H NMR of aromatic region of diethylamine reaction before being high vacuumed to remove excess amine. The unknown aromatic impurities are highlighted.

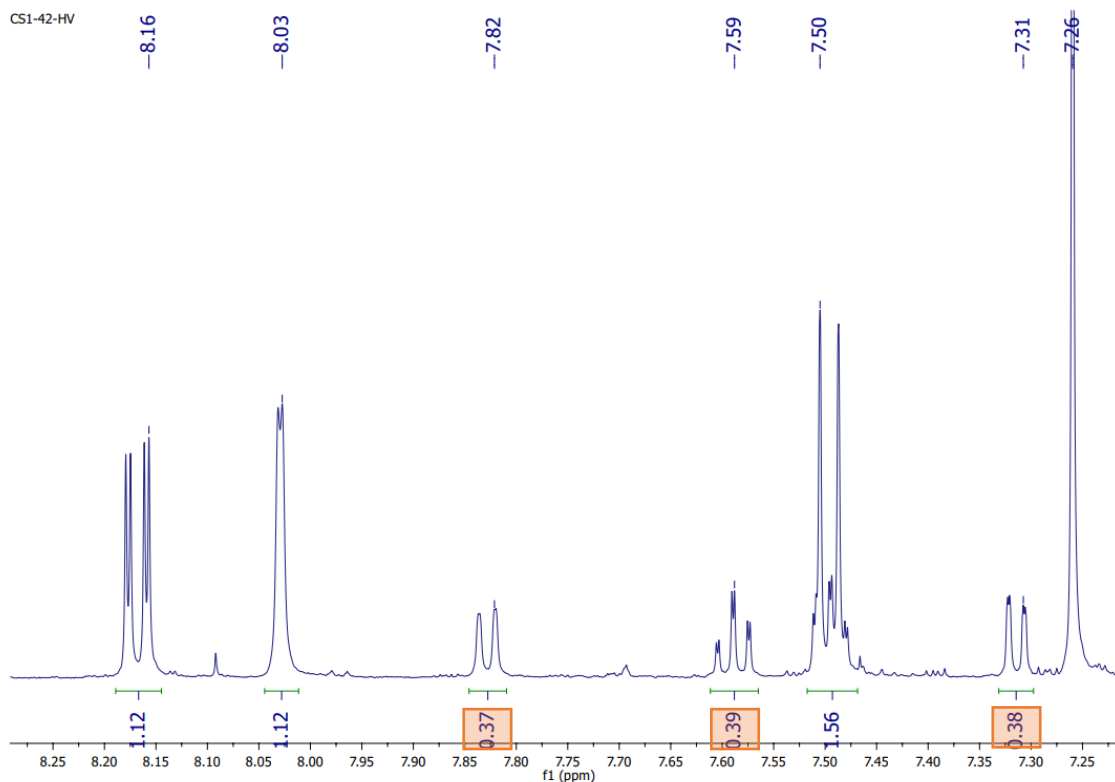


Figure 10. Zoomed in ^1H NMR of aromatic region of diethylamine reaction after being high vacuumed to remove excess amine. The unknown aromatic impurities are highlighted.

R_f – 0.14, 5% methanol in DCM with triethylamine, UV light

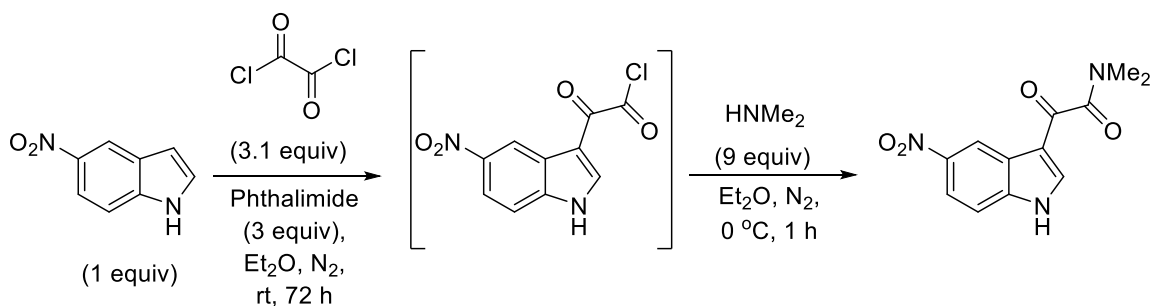
^1H NMR (500 MHz, Acetone- d_6) δ 9.16 (d, $J = 2$ Hz, 1H), 8.34 (s, 1H), 8.19 (dd, $J_s = 2.5$ Hz^{iv}, $J_f = 9$ Hz^v, 1H), 7.80 (dd, $J_s = 0.5$ Hz, $J_f = 9$ Hz, 1H), 3.52 (q, $J = 7.2$ Hz, 2H), 3.39 (q, $J = 7.2$ Hz, 2H), 1.24 (t, $J = 7.5$ Hz, 3H), 1.18 (t, $J = 7$ Hz, 3H).

^{12}C NMR (500 MHz, Acetone- d_6) δ 186.68, 166.54, 143.85, 140.09, 139.12, 125.04, 118.87, 117.77, 115.36, 113.06, 41.99, 38.67, 13.80, 12.21.

^{iv} J_s is the average J value determined for the peaks that are closest together in the doublet of doublets

^v J_f is the average J value determined for the peaks that are farthest apart in the doublet of doublets

Synthesis of Indole Intermediate with dimethylamine

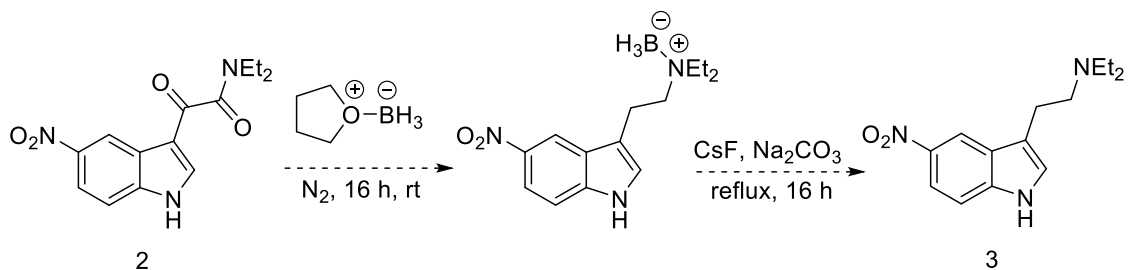


Scheme 7. Dimethylamine reaction to produce compound 2.

5-nitroindole 107.2 mg (0.6611 mmol) was added to a 25 mL pear flask. Next, 341.4 mg (1.84 mmol) of phthalimide and 8 mL of diethyl ether were added to the pear flask. The flask was then flushed with nitrogen and then 0.15 mL (1.749 mmol) of oxalyl chloride was added to the flask. The contents were stirred for 72 hours at room temperature. Reaction progress was monitored using TLC using 10% acetone in DCM. After 72 hours, 8 mL of diethyl ether was added to the flask. The reaction was then placed in an ice bath and flushed with nitrogen while 0.3 mL (4.47 mmol) of diethylamine were added. The reaction was stirred at 0 °C for 1 hour. The resulting yellow mixture was extracted using DCM and water three times. pH adjustment was not needed, because the reaction was already at pH 3. The reaction was then washed with a 0.6 M sodium bicarbonate solution three times. The liquid solution was dried using MgSO_4 and concentrated in a rotary evaporator to produce a crude, yellow/orange solid. Column chromatography was not run on this dimethylamine reaction or the following dimethylamine reactions. Column chromatography was run on the two previous dimethylamine reactions in 5% acetone in DCM for one reaction and 1:1 DCM: Hexanes for the second.

^1H NMR (500 MHz, Acetone- d_6) δ 9.22 (d, J = 2 Hz, 1H), 8.10 (d, J = 2.5 Hz, 1H), 7.56 (q, J = 4.7 Hz, 1H), 7.36 (s, 1H), 3.64 (s, 3H), 3.19 (s, 3H).

Reduction



Scheme 8. Reduction of compound 2 to compound 3.

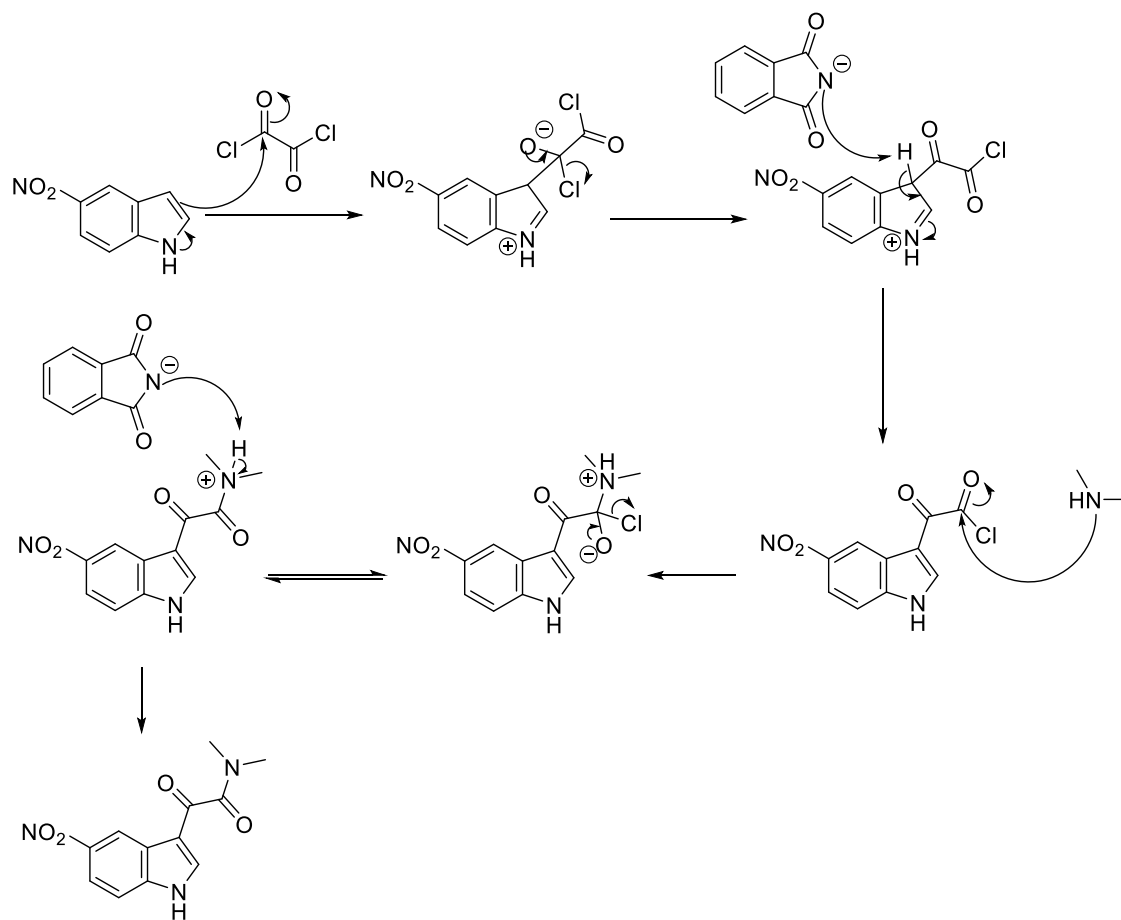
Compound 2 (28.6 mg, 0.098 mmol) was transferred to a round bottom flask and placed under nitrogen. To this, 1.5 mL of borane tetrahydrofuran (1.5 mmol) was added. The reaction was stirred at room temperature for 16 hours. After 16 hours, the reaction was extracted in sodium bicarbonate and ether. The liquid solution was concentrated in a rotary evaporator. Reaction was checked using TLC in 5% acetone in DCM. ^1H NMR was taken on the product in deuterated acetone. Column chromatography was done in 5% acetone in DCM. Fractions were concentrated in a rotary evaporator. This reaction did not work, as seen in the NMR (Appendix). Also, during this time reaction conditions were still being worked out.

R_f –0.43, 5% acetone in DCM, UV light

CHAPTER 3

RESULTS, DISCUSSION, CONCLUSION, & FUTURE WORK

Results & Discussion



Scheme 9. Proposed mechanism for the formation of the dimethylamine moiety

A plausible mechanism (Scheme 9) for the first two steps in the synthesis of the indole intermediate (Scheme 5) is seen above. The nitrogen on the indole acts as an enamine and pushes electrons to the 3rd position carbon. This carbon then acts as the nucleophile and attacks the C-O bond in oxalyl chloride forming a tetrahedral intermediate. The electrons on the oxygen push down and push off chlorine as the leaving group.

Phthalimide deprotonates the hydrogen at the 3rd position, reforming the double bond and pushing the electrons back onto the nitrogen. Then the lone electrons on the amine attack the other C-O bond forming another tetrahedral intermediate. The electrons on the oxygen again push down, reforming the carbonyl and pushing off chlorine as the leaving group. The proton on the nitrogen is removed by phthalimide.

Diethylamine and dimethylamine reactions to produce compound 2

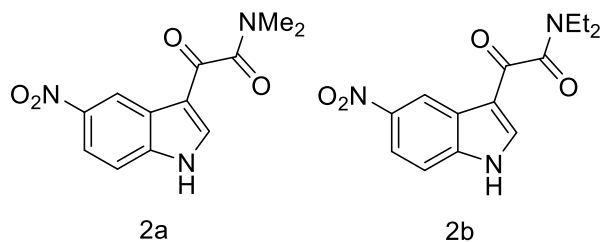


Figure 11. Compound 2

For the diethylamine reaction (Scheme 6) and dimethylamine reaction (Scheme 7), the desired product was compound two (Figure 11). This compound was successfully produced and isolated in one diethylamine reaction, but failed to be isolated again in future reactions. The difficulties lied in separation of impurities using column chromatography. The column chromatography conditions for the first reaction were 5% methanol in DCM with triethylamine which was then increased to 10% methanol in DCM. Column chromatography conditions were varied throughout the experiments in hopes to get the best separation. One solvent system used for another diethylamine reaction was 5% acetone in DCM that was increased to 11% acetone in DCM. Triethylamine was also added to the 5% acetone in DCM system in an attempt to make the column run faster. It was believed that by increasing the polarity of the solvent system, then the separation would be better. Meaning that the amine would not be present in all of the collected fractions. However,

this solvent system seemed to be too polar since good separation was not achieved. The poor separation had to do with amine streaking throughout the column. There would continuously be excess amine present throughout the collected fractions (Figure 12). Another difficulty in separation is that the product would often stick to the column, resulting in the polarity continuously being increased. In a later reaction, a less polar solvent system was used in an attempt to improve separation in comparison to the last system. This solvent system started with 100% DCM and was increased to 10% acetone in DCM. A large amount of color was observed coming off the column during the switch from 5% to 10% acetone in DCM, indicating that an incremental increase should be done in the future. The solvent systems used in column chromatography were tested using TLC to see which yielded the best separation. The polarity of the solvent systems was increased in an attempt to move the product spot off of the baseline.

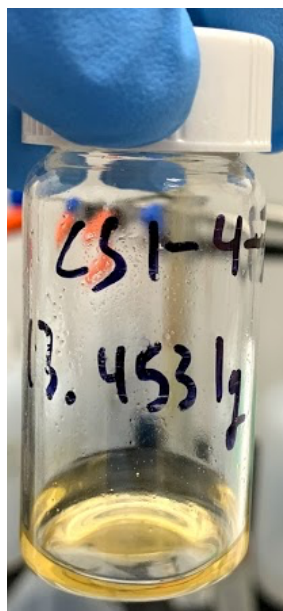


Figure 12. Vial containing product collected from a diethylamine reaction. The collected substance is a yellow liquid. The presence of excess liquid amine is evident here.

Another difficulty while purifying the reactions is the production of a yellow solid during the DCM/DI water extractions (Figure 13). This solid would start off in the organic layer, as can be seen in the figure, but would move to the aqueous layer once the pH was adjusted to 3 and the separatory funnel was shook. It remained a solid in the aqueous layer, indicating that it was insoluble in both phases. However, once it was in the aqueous layer it was discarded as waste. The solid was neither verified to be product or waste.



Figure 13. Image of a separatory funnel containing product being extracted in DCM and DI Water.

For both the diethylamine and dimethylamine experiments, reaction conditions were varied in hopes of optimizing the reaction yield. A summary of these conditions can be seen in Table 2. The reported overall yield of the reaction is 19%²⁷. To increase the yield

of product collected, the amount of starting material (5-nitroindole) was increased. The first few diethylamine and dimethylamine reactions used 30-40 mg of 5-nitroindole. This was increased to 100 mg to increase the yield of the reactions. Dimethylamine was used because it was the reported reagent used in the literature²⁷. Diethylamine was used in this experiment to vary the groups on the third position of the indole. For the majority of the reactions, the equivalencies used for oxalyl chloride and amine followed the reported equivalencies (approximately 3 and 9 equivalencies respectively). These were changed for the last three reactions due to very little product being produced, indicated by the low intensity of the product peak in ¹H NMR, and multiple unknown peaks showing up in ¹H NMR. At first just the equivalency of oxalyl chloride was increased because it was a plausible point of failure in the reaction due to the instability of the reagent over time and the age of the reagent bottle. The equivalency was increased by a factor of four to 12 equivalencies, while keeping all of the other reagents the same. This was because the first step of the reaction was not being completed which was indicated by unreacted starting material present in ¹H NMR (Figure 14). By increasing the amount of oxalyl chloride in the reaction, then more starting material would be reacted with to produce more product. However, it was realized that product wouldn't be produced if there wasn't enough amine present in the reaction to react with the oxalyl chloride. To fix this, the stoichiometry of phthalimide and amine were increased by a factor of four, while keeping starting material the same. The crude ¹H NMRs for all three of these reactions indicated the presence of product due to a product peak around 9 ppm. An example of the peak around 9 ppm can be seen in Figure 15.

Table 2. A summary of the reaction conditions for the various reactions ran.

Experiment	5-Nitroindole (mg, equiv)	Phthalimide (equiv)	Oxalyl Chloride (equiv)	Ether (mL) (step 1)	Ether (mL) (step 2)	Dimethylamine (equiv)	Diethylamine (equiv)	Percent Conversion
CS1-4	30, 1	-	3.1	2	1-1.5	-	9.3	-
CS1-9	30, 1	-	3.1	2	1-1.5	-	12.4	-
CS1-35	100, 1	3	3.1	5	2.5-3.75	-	9	-
CS1-42	100, 1	3	3.1	10	3	-	9	-
CS1-54	100, 1	3	3.1	8	8	-	9	41.15
CS1-63	100, 1	3	3.1	8	5	-	9	-
CS1-73	100, 1	12	12	10	5	-	36	5.51
CS1-19	30, 1	-	3.1	2	1-1.5	9.3	-	-
CS1-29	40, 1	2.8	3.1	2	1-1.5	9.3	-	-
CS1-60	100, 1	3	3.1	8	8	9	-	11.71
CS1-68	100, 1	3	12	10	6	9	-	-
CS1-71	100, 1	12	12	10	5	36	-	52.83

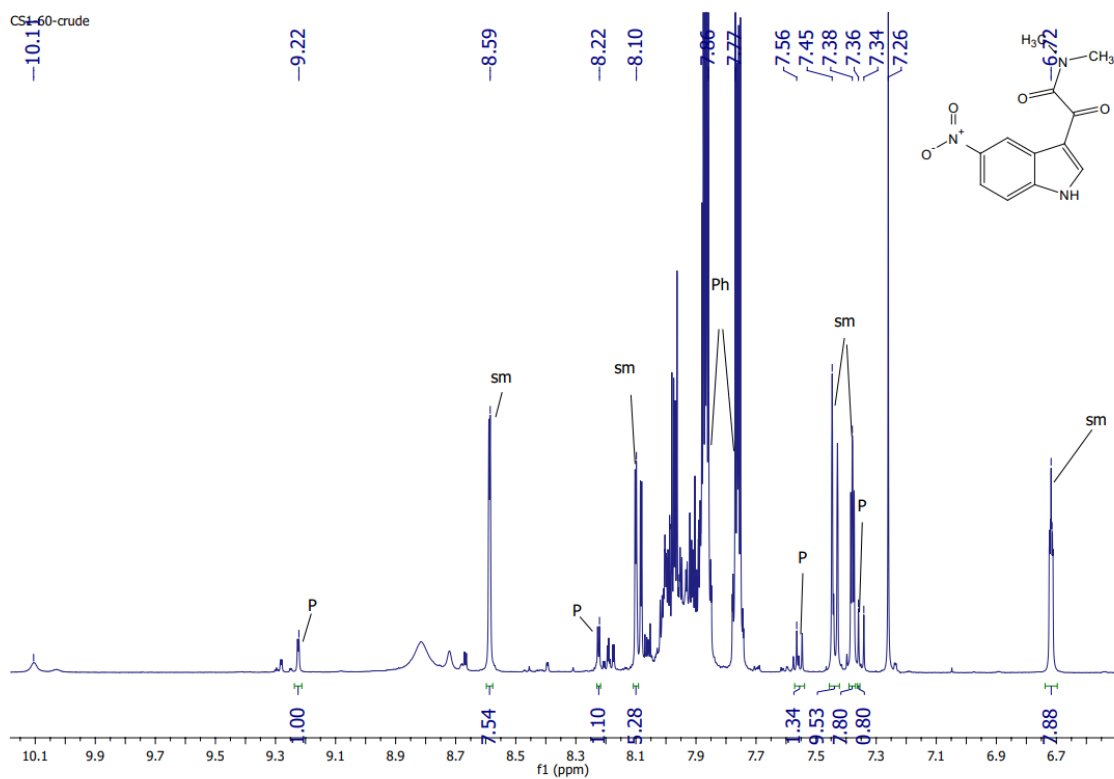


Figure 14. Zoomed in aromatic region in ¹H NMR of crude dimethylamine reaction showing the presence of unreacted starting material (sm).

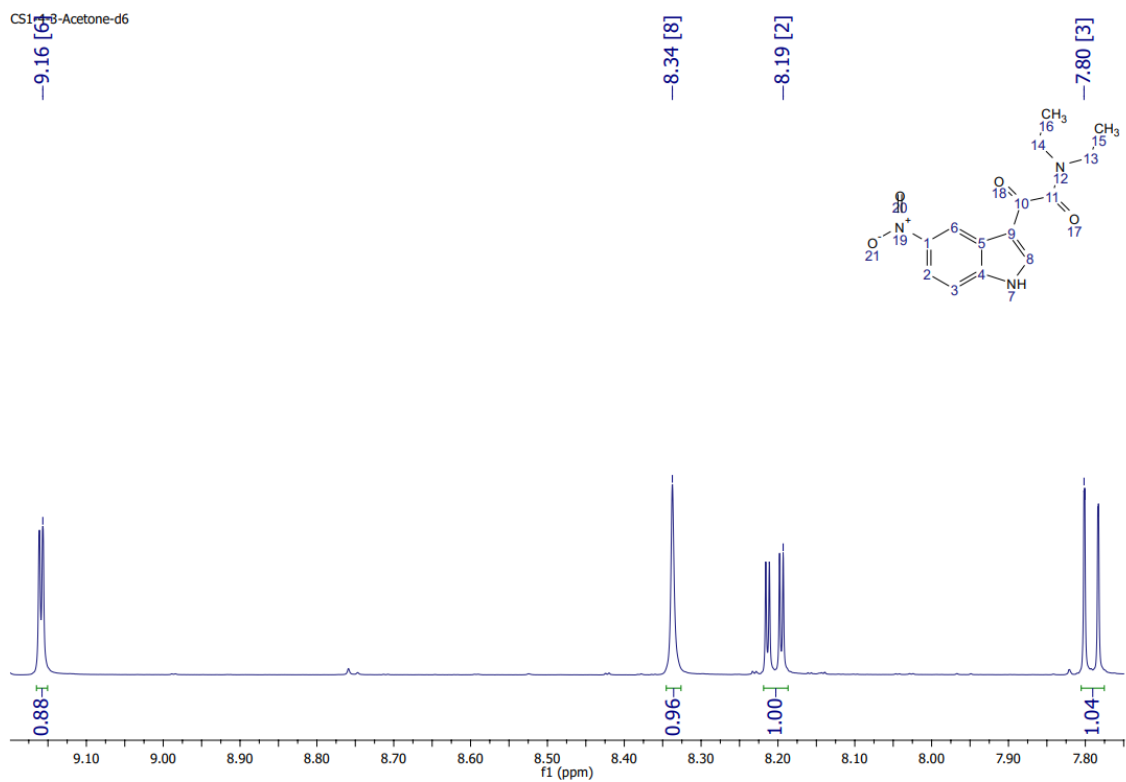
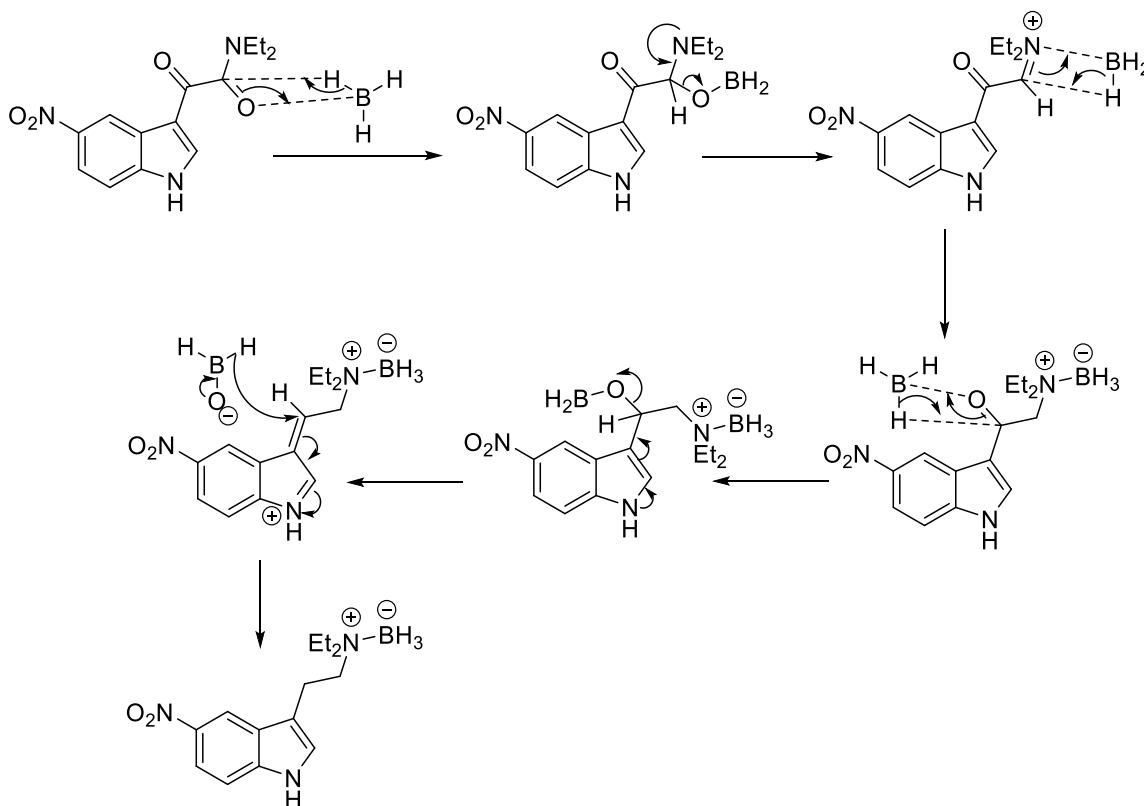


Figure 15. Zoomed in NMR of the diethylamine reaction to produce compound 2. The peak that indicates the presence of product is seen at 9.16 ppm.

Reduction of compound 2 to compound 3.



Scheme 10. The proposed mechanism for the diethylamine reduction of compound 2 to the borane-amine complex. This complex is further reacted with CsF and Na₂CO₃ to decompose the complex.

The proposed mechanism for the diethylamine reduction of compound 2 to compound 3 is seen in scheme 10. Borane reacts with one of the carbonyls to form a boron-oxygen bond and a carbon-hydrogen bond. Another equivalent of borane is required to react with the oxygen again and remove it and to give the carbon another hydrogen. This same process is repeated on the second carbonyl to produce compound 3.

For this reaction, borane tetrahydrofuran was added directly to compound 2 and then was worked up. However, the literature shows that a tryptamine-borane complex is formed once borane tetrahydrofuran is added to compound 2²⁷. The literature used cesium fluoride and sodium carbonate in an ethanol reflux to decompose the complex²⁷. This

would be done for future reactions. ^1H NMR was taken on the product in deuterated acetone. The NMR lacks the identifying peaks in the aromatic region for the production of compound 3, indicating that the reaction did not work (Figure 16). One of these key peaks would be a doublet at approximately 9 ppm. However, the spectrum below only shows one singlet at 8.14 ppm and two quartets around 7.70 ppm. Neither of these indicate the formation of product.

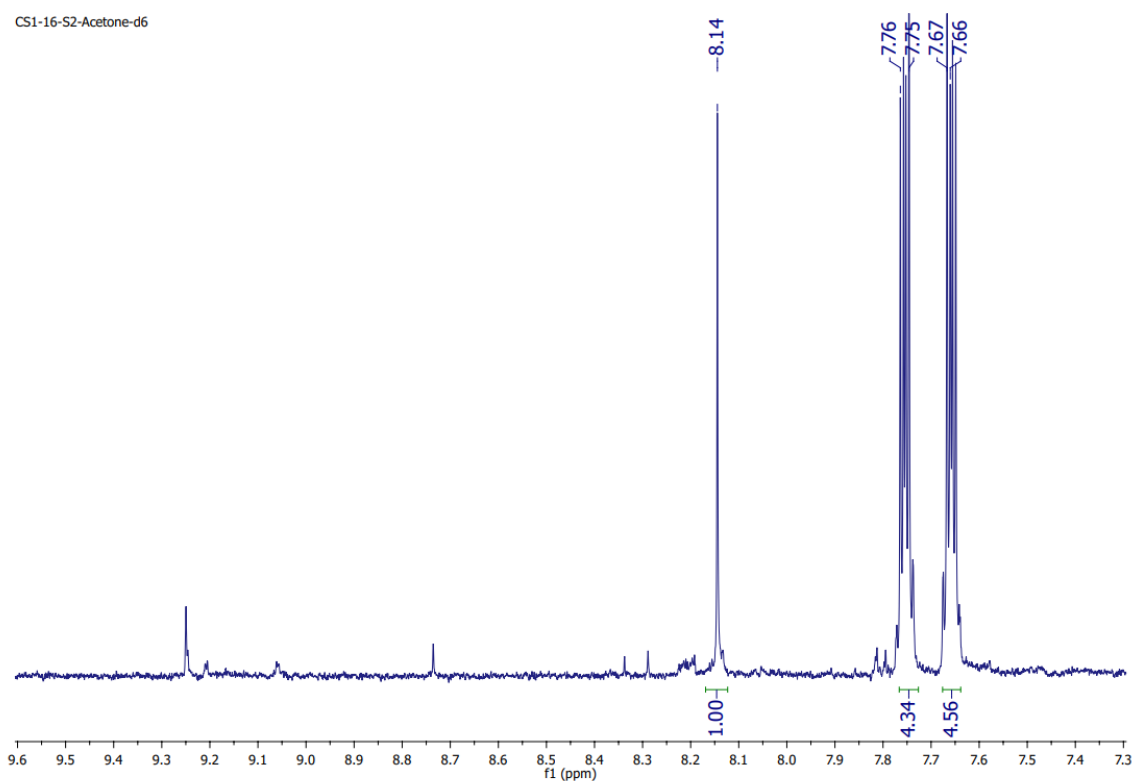


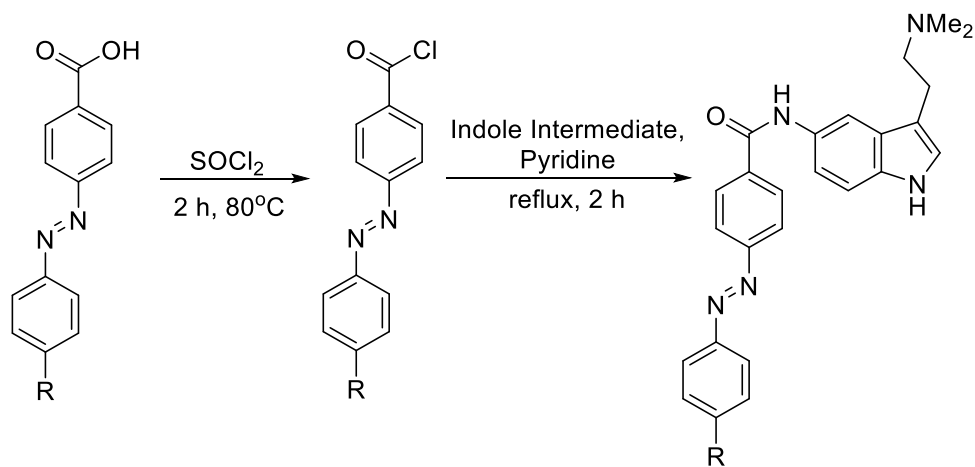
Figure 16. The zoomed in ^1H NMR of the reduction of compound 2 to produce compound 3.

Conclusion

Based on the ^{13}C and ^1H NMR spectra the first two steps of the synthesis of the indole intermediate were completed and product was produced with 72.9% yield. The column conditions that provided the best separation was determined to be 5% methanol in DCM with triethylamine, used in the diethylamine reaction to produce compound 2. The product isolated from this reaction seemed to have the cleanest NMR spectra. The reaction stopped working due to the age and instability of the reagents. The difficulty with the separation/purification steps was due to the solvent system being changed from the one used initially.

Future Work

Future work would be to finish this synthesis (Scheme 11), purify the product, and run ^{13}C and ^1H NMR and HRMS. To finish the synthesis, the reduction of the carbonyl on compound 2 using borane tetrahydrofuran, cesium fluoride, sodium carbonate, and an ethanol reflux to afford compound 3 needs to be performed. Then the reduction of the nitro group on compound 3 to afford compound 4 (indole intermediate) using hydrogen and palladium on carbon. Then azologization of the indole intermediate would be done through the Mills reaction seen in Scheme 12 or through the reaction seen in Scheme 13. In the Mills reaction, nitrosobenzene (commercially available) will be reacted with the indole intermediate to produce the first azo product. In the second reaction, 4-Phenylazobenzoic acid (commercially available) reacts with thionyl chloride to produce the azobenzene moiety that will react with the indole intermediate to produce the second azo product. Various substitutions could be induced onto the benzene rings to change the properties of the compounds. These products would be purified and their photochemical properties need to be verified. The product needs to be checked to determine if it is still a photoswitch and



Scheme 13. The second reaction to attach an azobenzene to the indole intermediate²¹. Where the R group represents the various substitutions that can occur on the benzene ring.

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APPENDIX

NMR SPECTRA

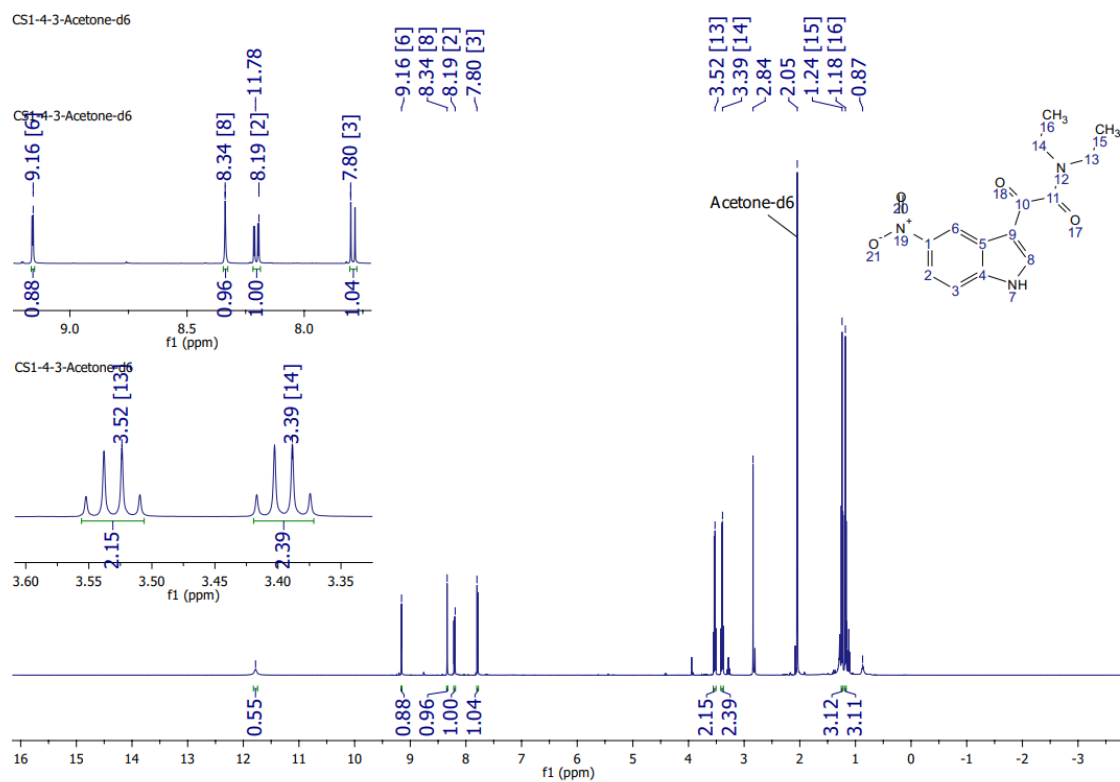


Figure 17. ^1H NMR (500 MHz, Acetone- d_6) spectrum for the first diethylamine reaction to produce compound 2.

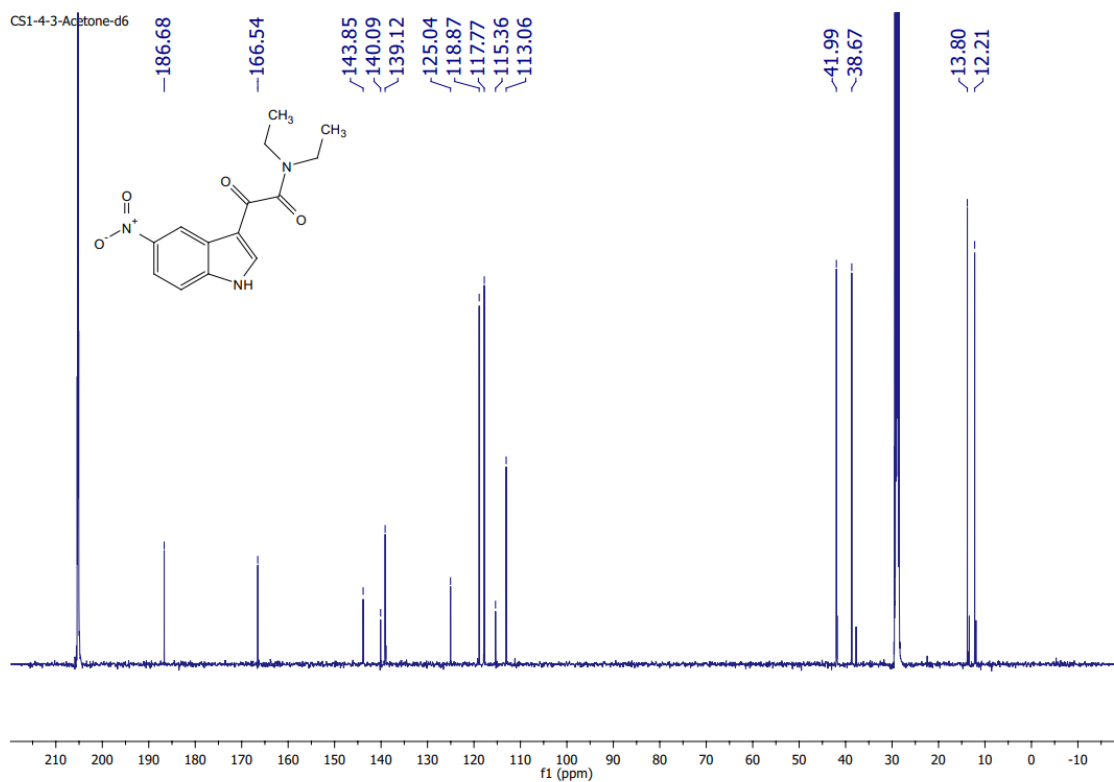


Figure 18. ¹³C NMR (500 MHz, Acetone-d₆) spectrum for the first diethylamine reaction to produce compound 2.

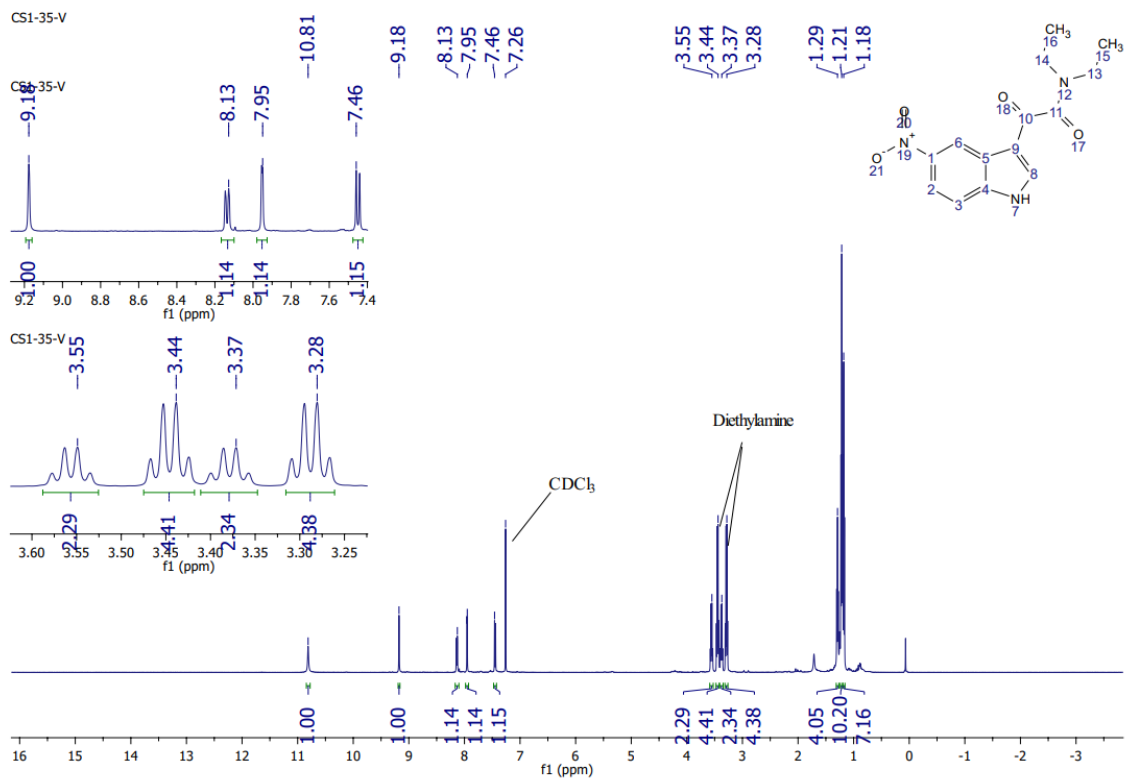


Figure 19. ¹H NMR (500 MHz, CDCl₃) spectrum for a later diethylamine reaction to produce compound 2.

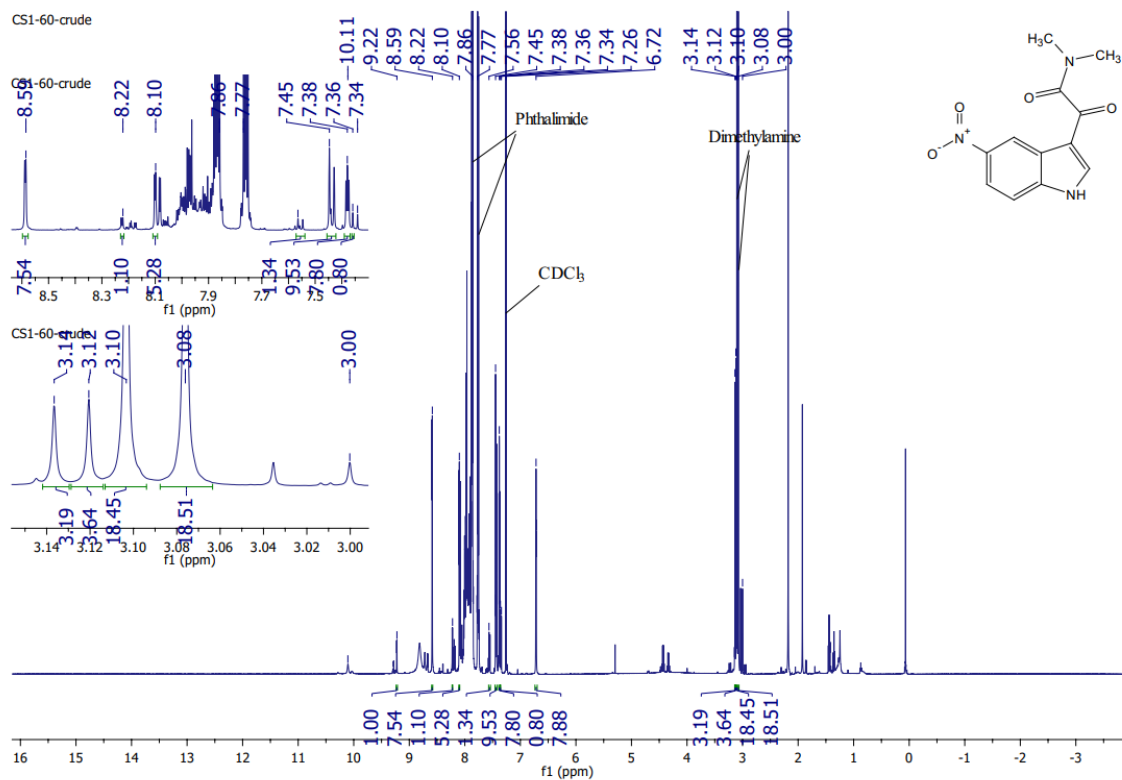


Figure 20. Crude ¹H NMR (500 MHz, CDCl₃) spectrum for a dimethylamine reaction to produce compound 2.

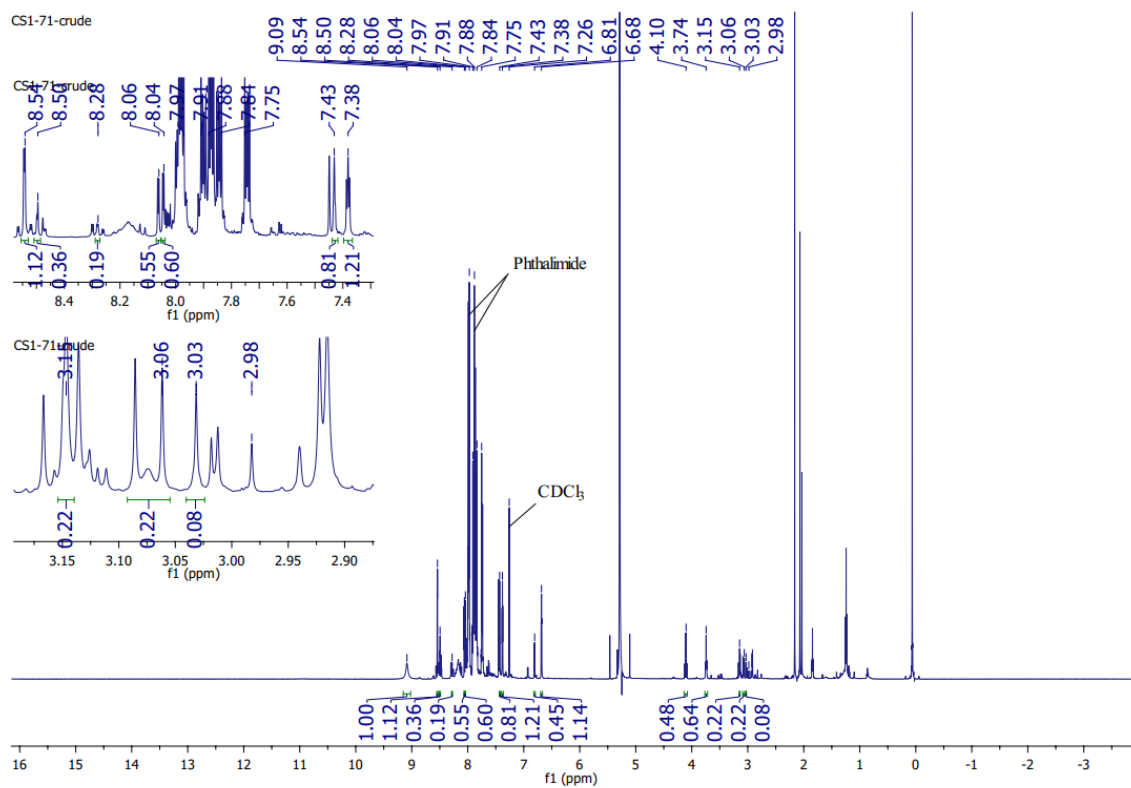


Figure 21. Crude ^1H NMR (500 MHz, CDCl_3) spectrum for a dimethylamine reaction to produce compound 2.

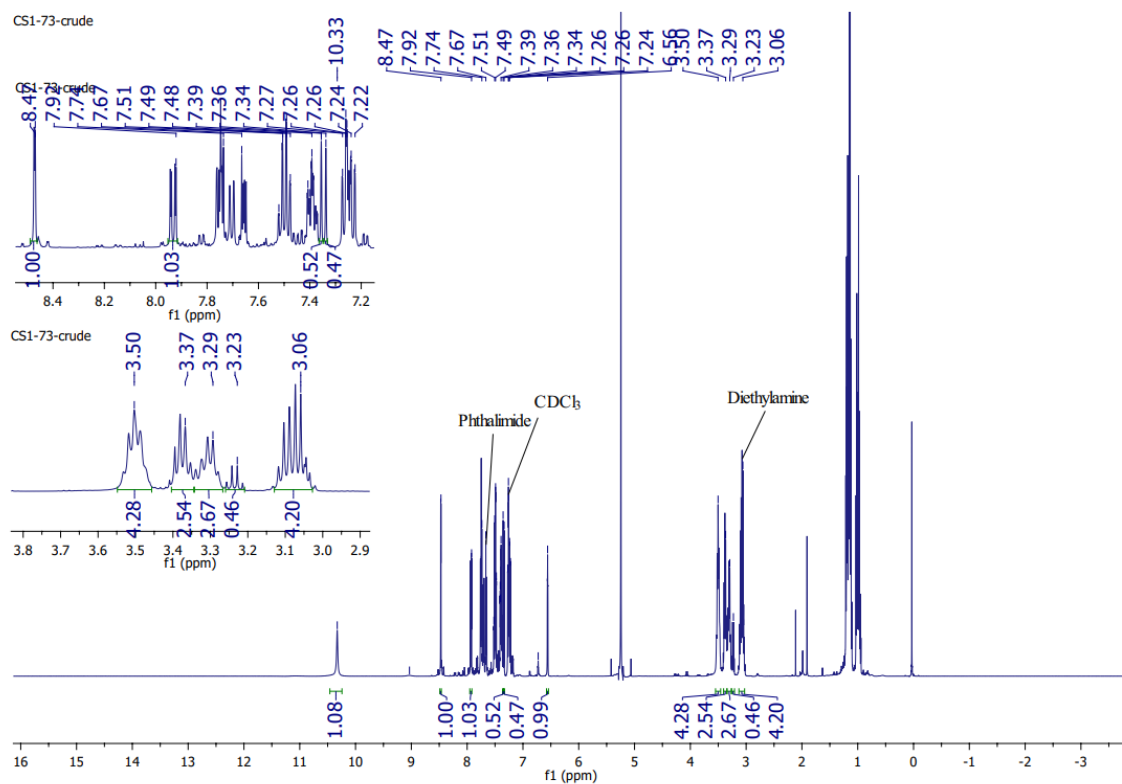


Figure 22. Crude ^1H NMR (500 MHz, CDCl_3) spectrum for the last diethylamine reaction to produce compound 2.

CS1-16-S2-Acetone-d6

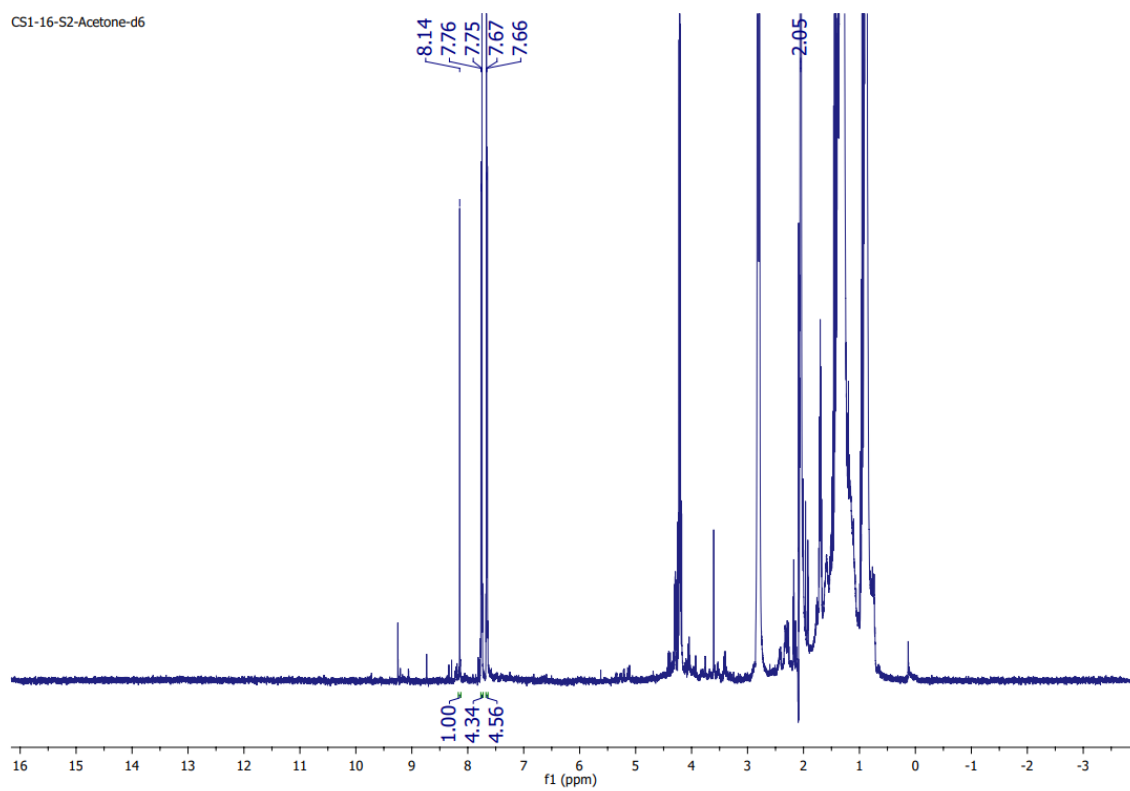


Figure 23. ^1H NMR (500 MHz, Acetone- d_6) spectrum for the reduction reaction of compound 2 to compound 3.

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Chelsea M Sainsbury was born in Danbury, Connecticut on October 16th, 1999. She was raised in Watertown, Connecticut and graduated from Watertown High School in 2017. Chelsea is majoring in chemistry.

After graduating, Chelsea will be working towards a PhD in chemistry at the University of Washington.